

IN THE SPECIFICATION:

Please insert the enclosed paper copy of the Sequence Listing after the last page of the specification after the Abstract.

Please replace the paragraph beginning on page 6, line 13 and ending on line 17 with the following paragraph:

Thus this invention provides a (poly)peptide comprising a T-cell epitope obtainable from the minor Histocompatibility antigen HA-1 comprising the sequence VLXDDLLEA (SEQ ID NO. [] 1) or a derivative thereof having similar functional or immunological properties, wherein X represents a histidine (H) or an arginine (R) residue.

Please replace the paragraph beginning on page 7, line 11 and ending on line 16 with the following paragraph:

A preferred embodiment of the invention is the peptide with the sequence VLHDDLLEA (SEQ ID NO. [] 2) that induces lysis of the cell presenting it at a very low concentration of peptide present. This does not imply that peptides inducing lysis at higher concentrations are not suitable. This will for a large part depend on the application and on other properties of the peptides, which were not all testable within the scope of the present invention.

Please replace the paragraph beginning on page 20, line 3 and ending on line 22 with the following paragraph:

As described above, in general, peptides presented in the context of HLA vary in length from about 7 to about 15 amino acid residues, and a polypeptide can be enzymatically processed to a peptide of such length. A peptide comprising HA-1 antigen provided by the invention typically is at least 7 amino acids in length, but preferably at least 8 or 9 amino acids. The upper length of a peptide provided by the invention is typically no more than 15 amino acids, but preferably no more than about 11 to 13 amino acids in length. A peptide provided by the invention contains the necessary anchoring residues for presentation in the groove of the relevant

HLA molecule. An immunogenic polypeptide provided by the invention comprises a 7-15 amino acid long peptide, optionally flanked by appropriate enzymatic cleavage sites allowing processing of the polypeptide. Presentation of the HA-1 antigen by MHC-I can occur in various ways depending on the particular type of MHC-I. Different HLA molecules behave differently in their capacity to present a peptide. In the present invention HA-1^H antigen can be presented by different HLA molecules. In case of HLA-A2 the peptide presented comprises the sequence VLHDDLLEA (SEQ ID NO:[] 2). When the HLA molecule is HLA-B60 the HA-1^H antigen comprises a sequence that is shifted slightly when compared to the sequence presented by HLA-A2. This is described in more detail below. However, the polymorphism is of course still present in the peptide presented by HLA-B60. Thus the HA-1 antigen may comprise any peptide capable of being presented by an MHC-I or for that matter MHC-II molecule provided that it comprises the relevant polymorphism.

Please replace the paragraph beginning on page 23, line 6 and ending on line 11 with the following paragraph:

We have found such further HA-1 peptides. A novel HLA-B60 restricted T cell epitope of HA-1 is provided comprising the sequence KECVLXDDL (SEQ ID NO:[] 3). X represents either a histidine or an arginine residue. With the HLA-B60 restricted HA-1 epitope it has become possible to enlarge the patient population for HA-1 specific immunotherapy. Patients that are negative for HLA-A2.1 but positive for HLA-B60 can now be subjected to a method of the invention.

Please replace the paragraph beginning on page 23, line 12 and ending on line 24 with the following paragraph:

In one aspect, the invention therefore provides a peptide constituting a T-cell epitope obtainable from the minor Histocompatibility antigen HA-1 comprising the sequence KECVLXDDL (SEQ ID NO:[] 3) or a derivative thereof having similar functional or immunological properties, wherein X represents a histidine or an arginine residue. The way these sequences are obtained is described in the examples. Nonameric as well as decameric peptides

have been found that show strong binding capacity to HLA-B60 molecules. Especially, the nonameric and decameric HA-1^{H/R} peptides KECVLHDDL (SEQ ID NO:[] 4), KECVLRDDL (SEQ ID NO:[] 5), KECVLHDDL (SEQ ID NO:[] 6) and KECVLRDDL (SEQ ID NO:[] 7) show strong binding to HLA-B60 molecules. Hence, in one embodiment a peptide or a derivative of the invention is provided, wherein the peptide comprises the sequence KECVLXDDL (SEQ ID NO:[] 8). As used herein, an X in a sequence of a peptide of the invention represents a histidine or an arginine residue.

Please replace the paragraph beginning on page 24, line 22 and ending on line 26 with the following paragraph:

Thus, in one aspect an immunogenic polypeptide obtainable from the minor Histocompatibility antigen HA-1 comprising the sequence KECVLXDDL (SEQ ID NO:[] 3) or a derivative thereof having similar functional or immunological properties is provided. In one embodiment the polypeptide comprises the sequence KECVLXDDL (SEQ ID NO:[] 8).

Please replace the paragraph beginning on page 24, line 30 and ending on page 25, line 4 with the following paragraph:

In a preferred embodiment of the invention, a peptide comprising the sequence KECVLHDDL (SEQ ID NO:[] 4) or KECVLHDDL (SEQ ID NO:[] 6) is provided, which induces lysis of the cell presenting it at a very low concentration of peptide present. This does not imply that peptides inducing lysis at higher concentrations are not suitable. This will for a large part depend on the application and on other properties of the peptides, which were not all testable within the scope of the present invention.

Please replace the paragraph beginning on page 25, line 18 and ending on line 31 with the following paragraph:

Further applications of a peptide, derivative and/or analog of the invention lie in the prophylactic administration to transplanted individuals to prevent GvHD. This is done with either

agonists, possibly in combination with an adjuvant, or with antagonists which block the responsible cells. This can be done with or without the concomitant administration of for instance TCR derived peptide sequences or cytokines. Furthermore, a peptide, derivative and/or analog of the invention is used to prepare a therapeutic agent capable of eliminating a subset of cells, directly or indirectly, especially cells of hematopoietic origin and/or tumor cells, as described above for HLA-A2.1 restricted peptides of the invention comprising the sequence VLXDDLLEA (SEQ ID NO:[] 1). The applications for an HLA-A2.1 restricted peptide of the invention, particularly suitable for HLA-A2.1 positive individuals, are similar to the applications of an HLA-B60 restricted peptide of the invention which are particularly suitable for HLA-B60 positive individuals. HA-1-specific immunotherapy can now be applied to HLA-A2.1 positive as well as HLA-B60 positive patients.

Please replace the paragraph beginning on page 26, line 24 and ending on line 27 with the following paragraph:

Preferably, the peptide or polypeptide of the invention comprises a peptide constituting a T-cell epitope obtainable from HA-1 comprising the sequence KECVLXDDL (SEQ ID NO:[] 3) or KECVLXDDL (SEQ ID NO:[] 8) or a derivative thereof having similar functional or immunological properties.

Please replace the paragraph beginning on page 32, line 13 and ending on line 18 with the following paragraph:

FIG. 2. Sequencing of mH HA-1 peptide by tandem mass spectrometry. a. Collision activation dissociation mass spectrum of peptide candidate (SEQ ID NO: 14) with m/z of 513. b. Reconstitution assay with different concentrations of synthetic mH HA-1 peptide with three HA-1 specific T cell clones, 3HA15, clone 15 and 5W38. Background lysis of T2 by the CTL in the absence of any peptide was for 3HA15 4%, for clone 15 10% and for 5W38 2%. Positive control lysis was for 3HA15 46%, for clone 15 47% and 5W38 48%.

Please replace the paragraph beginning on page 33, line 5 and ending on line 16 with the following paragraph:

FIG. 4. a. Binding of HA-1^H and HA-1^R peptides to HLA-A2.1. The binding of HA-1^H and HA-1^R peptides were assayed for their ability to inhibit the binding of fluorescent peptide FLPSDCFPSV (SEQ ID NO: 9) to recombinant HLA-A2.1 and b2-microglobulin in a cell free peptide binding assay. One representative experiment is shown. The IC₅₀ is determined on the results of 4 experiments and was 30 nM for VLHDDLLEA (SEQ ID NO: [] 2) and 365 nM for VLRDDLLEA (SEQ ID NO: [] 10). Also shown is YIGEVLVSV (SEQ ID NO: 77). b. Reconstitution assay with different concentrations of synthetic HA-1^R peptide with HA-1 specific T cells. The HA-1^R peptide was titrated and preincubated with T2 cells. Three HA-1 specific T cell clones, 5W38, 3HA15 and clone 15 were added and a 4 hr ⁵¹Cr-release assay was performed. Background lysis of T2 by the CTL in the absence of any peptide was for 3HA15 4%, for clone 15 10% and for 5W38 2%. Positive control lysis was for 3HA15 46%, for clone 15 47% and 5W38 48%.

Please replace the paragraph beginning on page 35, line 5 and ending on line 9 with the following paragraph:

FIG. 13. Binding of HA-1^{H/R} peptides to HLA-A3. The results are expressed as the percentage inhibition of the HLA binding of the 150 nM fluorescent reference peptide by the indicated peptides VLHDDLLEAR (SEQ ID NO: 43) and VLRDDLLEAR (SEQ ID NO: 44) added at serial dilutions (see material and methods). Curves were fitted by nonlinear regression and one site binding equation. The IC₅₀ value of the HLA-A3 binder positive control peptide KQSSKALQR (9) (SEQ ID NO: 11) was 9.4 μM.

Please replace the paragraph beginning on page 35, line 10 and ending on line 14 with the following paragraph:

FIG. 14. Efficient binding of HA-1^{H/R} peptides to HLA-B60. The results are expressed as the percentage inhibition of the HLA binding of the 150 nM reference peptide by the indicated peptides KECVLHDDL (SEQ ID NO: 4), KECVLRDDL (SEQ ID NO: 5), KECVLHDDL

(SEQ ID NO: 6), and KECVLRDDLL (SEQ ID NO: 7) added at serial dilutions (see material and methods). Curves were fitted by nonlinear regression and one site binding equation. The IC₅₀ value of the HLA-B60 binder positive control peptide KESTLHLVL (9) (SEQ ID NO: 12) was 1.1 μ M.

Please replace the paragraph beginning on page 35, line 15 and ending on line 19 with the following paragraph:

FIG. 15. Stable binding of nonameric and decameric HA-1^{H/R} peptides to HLA-B60. The nonameric and decameric HA-1^{H/R} peptides were tested for binding to HLA-B60 (a, b) KECVLHDDL (SEQ ID NO:6), KECVLHDDL (SEQ ID NO: 4) and KECVLRDDLL (SEQ ID NO: 7), KECVLRDDL (SEQ ID NO: 5) and to HLA-A2 (c) VLHDDLLEA (SEQ ID NO: 2) and VLRDDLLEA (SEQ ID NO: 10), at the indicated temperatures. The results are expressed as the percentage inhibition of HLA the binding of the reference peptide. Curves were fitted by nonlinear regression and one site binding equation.

Please replace the paragraph beginning on page 37, line 7 and ending on page 38, line 9 with the following paragraph:

To identify the mH antigen HA-1, HLA-A2.1 molecules were purified from two HA-1 expressing EBV-transformed B lymphoblastoid cell lines (EBV-BLCL) Rp and Blk. The HLA-A2.1 bound peptides were isolated by acid treatment and fractionation of the peptides was performed by multiple rounds of reverse phase HPLC. The fractions were analyzed for their capacity of inducing HA-1 specific lysis using T2 cells as target cells and an HA-1 specific CTL clone as effector cells in a ⁵¹Cr-release assay (FIG. 1a). Fraction 24 contained HA-1 activity and was two times further fractionated with reverse phase HPLC using a different organic modifier (FIG. 1b.c.). Fraction 33 and 34 of the third HPLC fractionation showed HA-1 activity ⁵¹Cr-release assay and were analyzed by tandem mass spectrometry. Because over a 100 different peptides were present in these fractions, around 40% of fractions 33 and 34 was chromatographed with an on-line microcapillary column effluent splitter. The fractions were simultaneously analyzed by tandem mass spectrometry and ⁵¹Cr-release assay (FIG. 1d.). Five

peptide species (at m/z 550, 520, 513, 585 and 502) were specifically present in active fractions and absent in fractions without activity in the CML assay. Collision activated dissociation analysis of peptide candidate m/z 550 revealed the sequence YXTDRVMTV (SEQ ID NO:[] 13). X stands for Isoleucine or leucine that cannot be discriminated with this type of mass spectrometer. However, a synthetic peptide with this sequence was not able to reconstitute the HA-1 epitope (results not shown). To determine which of the four remaining candidates was the HA-1 peptide the second HA-1 purification of the EBV-BLCL Blk was evaluated. HA-1 positive peptide fraction 33 of the second reverse phase HPLC fractionation was further chromatographed by microcapillary HPLC with a third organic modifier. A single peak of reconstituting activity was observed in a ⁵¹Cr-release assay (results not shown). Mass spectral analysis of these fractions revealed that only peptide candidate m/z 513 was present. This peptide was analyzed with collision activated dissociation analysis and sequenced as VXHDDX XEA (SEQ ID NO:[] 14) (FIG. 2a). Isoleucine and leucine variants of the peptide were synthesized and run on the microcapillary HPLC column. Only peptide VLHDDLLEA (SEQ ID NO:[] 2) coeluted with the naturally processed peptide 513 (results not shown). Next, synthetic VLHDDLLEA (SEQ ID NO:[] 2) added in different concentration to a CML assay with 3 different HA-1 specific CTL clones revealed recognition by all three clones of the peptide with a half maximal activity at 150-200 pM for or all three clones (FIG. 2b). This demonstrated that the mH antigen HA-1 is represented by the nonapeptide VLHDDLLEA (SEQ ID NO:[] 2).

Please replace the paragraph beginning on page 38, line 10 and ending on line 31 with the following paragraph:

Database searches performed to identify the gene encoding HA-1, revealed that the HA-1 peptide VLHDLLEA (SEQ ID NO:[] 15) was identical for 8 out of 9 amino acids with the peptide VLRDDLLEA (SEQ ID NO:[] 10) from the KIAA0223 partial complementary DNA (cDNA) sequence, derived from the acute myelogenous leukemia KG-1 cell line. Because HA-1 has a population frequency of 69%, we reasoned that VLRDDLLEA (SEQ ID NO:[] 10) might represent the HA-1 allelic counterpart present in the remaining 31% of the population. To elaborate on this assumption, we performed cDNA sequence analysis of the putative HA-1

encoding region of KIAA0223 in EBV-BLCL derived from a presumed HA-1 homozygous positive (vR), from a presumed HA-1 negative individual (DH) and from the KG-1 cell line (Table 6.). The HA-1 encoding region of KIAA0223 of the HA-1+/+ individual (vR) displayed two nucleotides differences from the KIAA0223 sequence in the databank, leading to the amino acid sequence VLHDDLLEA (SEQ ID NO:[] 2) (designated HA-1^H). The HA-1 encoding region of KIAA0223 of the HA-1-/- individual (DH) showed 100% homology with the reported KIAA0223 sequence (designated HA-1^R). The KG-1 cell line expressed both KIAA0223 alleles. Because KG-1 does not express the restriction molecule HLA-A2.1 necessary for T cell recognition, we transfected KG-1 with HLA-A2.1 and used these cells as target cells in a ⁵¹Cr-release assay with the HA-1 specific T cell clone as effector cells. According to the cDNA sequence analysis results, the KG-1 cells were recognized by the HA-1 specific T cell clone (data not shown). This result suggested that the KIAA0223 gene forms a di-allelic system of which the HA-1^H allele leads to recognition by the mH antigen HA-1 specific T cell clones.

Please replace the paragraph beginning on page 39, line 22 and ending on page 40, line 2 with the following paragraph:

Reconstitution and HLA-A2.1 binding assays were performed to determine the capacity of HA-1^R peptide VLRDDLLEA (SEQ ID NO:[] 10) to bind to HLA-A2.1 and to be recognized by the HA-1 specific T cell clones. The concentration of the HA-1^R peptide that inhibited the binding of a fluorescent standard peptide to HLA-A2.1 by 50 % (IC50) was 365 nM, falling in the intermediate binders, whereas the IC50 of the HA-1^H peptide was 30 nM, which is in the range of high affinity binders (FIG. 4a) ^{13,14}. Different concentrations of VLRDDLLEA (SEQ ID NO:[] 10) were tested in a ⁵¹Cr-release assay with three HA-1 specific T cell clones. One out of the three clones (3HA15) tested showed recognition of the HA-1^R peptide, but only at 1000 times higher peptide concentration than that necessary for the recognition of the HA-1^H peptide (FIG. 4b). As the binding affinity of the two peptides to HLA-A2.1 differs only 10-fold, it can be concluded that all the T cell clones specifically recognize the HA-1^H peptide.

Please replace the paragraph beginning on page 40, line 3 and ending on line 23 with the following paragraph:

The 3HA15 T cell clone, recognizing the HA-1^R peptide at high concentrations, does not recognize HA-1^R homozygous individuals. This suggests that VLRDDLLEA (SEQ ID NO:[] 10) is not presented by HLA-A2.1 or presented below the detection limit of the T cell. To determine whether the HA-1^R peptide VLRDDLLEA (SEQ ID NO:[] 10) was presented by HLA-A2.1, HLA-A2.1 bound peptides were eluted from an HA-1^R homozygous EBV-BLCL and fractionated with reverse phase HPLC. The synthetic HA-1- peptide VLRDDLLEA (SEQ ID NO:[] 10) was run on reverse HPLC to determine at which fraction this peptide eluted. The corresponding HPLC fractions derived from the HA-1^R expressing EBV-BLCL were analyzed using mass spectrometry. Presence of peptide VLRDDLLEA (SEQ ID NO:[] 10) could not be detected (results not shown), indicating that this peptide is not or in very low amounts presented by HLA-A2.1 on the cell surface. This is most likely due to the 10-fold lower binding affinity of the peptide for HLA-A2.1. The supposed absence of the HA-1^R peptide in HLA-A2.1 indicates that this allele must be considered as a null allele with regard to T cell reactivity. This implicates that only BMT from an HA-1^{R/R} (HA-1-) donor to HA-1^{H/H} or HA-1^{R/H} (HA-1+) recipient direction and not the reverse would be significantly associated with GvHD. This is indeed observed in a retrospective study in which HLA-A2.1 positive BMT pairs were typed for HA-1³. However, HA-1^R derived peptides may bind to other HLA alleles and possibly be recognized by T cells. If the latter peptides are not generated and presented by the HA-1^H allele, then T cell reactivity towards the HA-1^R allele may be envisaged and GvHD in that direction may occur.

Please replace the paragraph beginning on page 46, line 13 and ending on line 25 with the following paragraph:

HLA-A2.1 peptide binding assay. A quantitative assay for HLA-A2.1 binding peptides based on the inhibition of binding of the fluorescent labeled standard peptide Hbc 18-27 F to C6 (FLPSDCFPSV) (SEQ ID NO: 9) to recombinant HLA-A2.1 protein and b2-microglobulin was

used ^{26,27}. In short, HLA-A2.1 concentrations yielding approximately 40-60% bound fluorescent standard peptide were used with 15 pmol/well (150 nM) b2-microglobulin (Sigma). Various doses of the test peptides were coincubated with 100 fmol/well (1 nM) fluorescent standard peptide, HLA-A2.1 and b2-microglobulin for 1 day at room temperature in the dark in a volume of 100 µl in assay buffer. The percent of MHC-bound fluorescence was determined by gel filtration and the 50% inhibitory dose was deduced for each peptide using one-site competition non-linear regression analysis with the prism graph software. Synthetic peptides were manufactured on a Abimed 422 multiple peptide synthesizer (Abimed, Langenfeld, Germany) and were more than 90% pure as checked by reverse phase HPLC.

Please replace the paragraph beginning on page 46, line 26 and ending on page 47, line 8 with the following paragraph:

RT-PCR amplification and sequencing of KIAA0223 region coding for HA-1. Total or mRNA was prepared from BLCL using the RNazol method (Cinna/Biotech Laboratories, Houston, TX) or according to manufacturer's instructions (QuickPrep mRNA purification Kit, Pharmacia Biotech). CDNA was synthesized with 1 µg RNA as template and with KIAA0223 based reverse primer 5'-GCTCCTGCATGACGCTCTGTCTGCA-3' (SEQ ID NO:[] 16). To amplify the HA-1 region of KIAA0223 the following primers were used: Forward primer 5'-GACGTCGTCGAGGACATCTCCCAT-3' (SEQ ID NO:[] 17) and reverse primer 5'-GAAGGCCACAGCAATCGTCTCCAGG-3' (SEQ ID NO:[] 18). Cycle parameters used were denaturation 95 °C, 1 min, annealing 58 °C, 1 min and extension 72 °C, 1 min (25 cycles). The PCR-products were purified using the Magic PCR-Preps DNA purification System (Promega) and direct cloned using the pMosBlue T-vector kit (Amersham LIFE SCIENCE). Six independent colonies from each individual were sequenced using the T7-sequencing kit (Pharmacia Biotech).

Please replace the paragraph beginning on page 47, line 9 and ending on line 16 with the following paragraph:

HA-1 allele specific PCR amplification. In the case of HA-1 allele specific PCR amplification, cDNA was synthesized as described above. A PCR amplification was performed with allele specific forward primers: for the HA-1^H allele primer H1: 5'-CCT-TGA-GAA-ACT-TAA-GGA-GTG-TGT-GCT-GCA-3' (SEQ ID NO:[] 19), for the HA-1^R allele primer R1: 5'-CCT-TGA-GAA-ACT-TAA-GGA-GTG-TGT-GTT-GCG-3' (SEQ ID NO:[] 20) and for both reaction the reverse primer as described above was used. Cycle parameters used were denaturation 95 °C, 1 min, annealing 67 °C, 1 min and extension 72 °C, 1 min (25 cycles).

Please replace the paragraph beginning on page 47, line 17 and ending on line 30 with the following paragraph:

Cloning and expression of HA-1^H and HA-1^R allelic region of KIAA0223. A forward KIAA0223 based PCR primer containing an ATG start codon (5'-CCG-GCA-TGG-ACG-TCG-TCG-AGG-ACA-TCT-CCC-ATC-3' (SEQ ID NO:[] 21)) and a reverse KIAA0223 based PCR primer containing a translational stop signal (5'-CTA-CTT-CAG-GCC-ACA-GCA-ATC-GTC-TCC-AGG-3' (SEQ ID NO:[] 22)) were designed and used in a RT-PCR reaction with cDNA derived from an homozygous HA-1^H and a homozygous HA-1^R BLCL. Cycle parameters used were denaturation 95 °C, 1 min, annealing 60 °C, 1 min and extension 72 °C, 1 min (25 cycles). The desired PCR-products were purified using the Magic PCR-Preps DNA purification System (Promega). The purified DNA was direct cloned using the pMosBlue T-vector kit (Amersham LIFE SCIENCE) and recloned in the eukaryotic pCDNA3.1(+) vector under the control of a CMV promoter. Transient cotransfections were performed with HLA-A2.1 in Hela cells using DEAE-Dextran coprecipitation. After 3 days of culture HA-1 specific T cells were added and after 24 hours the TNFa release was measured in the supernatant using WEHI cells²⁸.

Please replace the paragraph beginning on page 55, line 24 and ending on page 56, line 12 with the following paragraph:

Effective binding of nonameric and decameric HA-1^H and HA-1^R peptides to HLA-B60.

Three categories of HLA molecules were selected for the peptide binding assays: those molecules with a frequency of more than 10 % in the Caucasian population, those with binding motifs and those that were predicted to bind nonameric / decameric HA-1^{H/R} peptides. All nonameric HA-1^H and HA-1^R peptides (n=18) were tested for binding to the so called frequent HLA class I molecules HLA-A1, -A2, -A3, -A11, -A24, -B7, -B8, -B35, -B62. The peptide analysis was extended with two decameric HA-1^{H/R} peptides with a binding motif for HLA-A3 and with five nonameric/decameric peptides predicted to bind either to HLA-B14 or -B60 (table 3). The HLA-A1, -A11, -A24, -B7, -B8, -B14, -B35 and -B62 molecules did not bind nonameric HA-1^{H/R} peptides, despite the predictions of BIMAS software for intermediate to strong binding of peptide ECVLRDDLL (SEQ ID NO: 23) to HLA-B8 or to -B14 (table 3). The decameric HA-1^{H/R} peptides VL^{H/R}DDLLEAR showed weak to intermediate binding to HLA-A3 molecules with IC₅₀ values of 15.6 µM and 37.5 µM respectively (FIG. 13). In agreement with the prediction of the BIMAS software, the nonameric and decameric HA-1^{H/R} peptides KECVLHDDL (SEQ ID NO: 4), KECVLRDDL (SEQ ID NO: 5), KECVLHDDL (SEQ ID NO: 6) and KECVLRDDL (SEQ ID NO: 7) showed strong binding to HLA-B60 molecules with very low IC₅₀ values of 5.3 µM, 3.9 µM, 1.0 µM and 1.6 µM respectively (FIG. 14). As expected, the original HLA-A2/HA-1^H CTL epitope, also predicted by the BIMAS software, displayed binding to HLA-A2 with an IC₅₀ value of 6.4 µM (data not shown).

Please replace the paragraph beginning on page 59, line 27 and ending on page 60, line 26 with the following paragraph:

Detection of disseminated cells, global amplification of micro- dissected areas and of single cells from bone marrow and lymph nodes was performed as described in detail (Klein et al., submitted). Briefly, the viable bone marrow or lymph node samples were stained for 10 min. with 10 µg/ml monoclonal antibody 3B10-C9 in the presence of 5% AB-serum. 3B10-C9-positive cells were detected with B-phycoerythrin-conjugated goat antibody to mouse IgG (The

Jackson Laboratory) and transferred to PCR-tubes on ice. Oligo-dT beads in 10 µl lysis buffer (Dynal) were added, the cells lysed, tubes rotated for 30 min. to capture mRNA. 10 µl cDNA wash buffer-1 (50 mM Tris-HCl, pH 8.3, 75mM KCl, 3mM MgCl₂, 10mM DTT, supplemented with 0.5% Igepal (Sigma)) was added and mRNA bound to the beads washed in 20 µl cDNA wash buffer-2 (50 mM Tris-HCl, pH 8.3, 75mM KCl, 3mM MgCl₂, 10mM DTT, supplemented with 0.5% Tween-20 (Sigma)), transferred to a fresh tube and washed again in cDNA wash buffer-1. mRNA was reverse transcribed with Superscript II Reverse Transcriptase (Gibco BRL) using the buffers supplied by the manufacturer supplemented with 500 µM dNTP, 0.25% Igepal, 30 µM CFL5c8 primer (5'-(CCC)5 GTC TAG ANN (N)8-3' (SEQ ID NO:[] 25)) and 15 µM CFL5cT (5'-(CCC)5 GTC TAG ATT (TTT)4 TVN (SEQ ID NO:[] 26), at 44°C for 45 min. Samples were rotated during the reaction to avoid sedimentation of the beads. cDNA remained linked to the paramagnetic beads via the mRNA and was washed once in the tailing wash buffer (50 mM KH₂PO₄, pH 7.0, 1mM DTT, 0.25% Igepal). Beads were resuspended in tailing buffer (10 mM KH₂PO₄, pH 7.0, 4 mM MgCl₂, 0.1 mM DTT, 200 µM GTP) and cDNA-mRNA hybrids were denatured at 94 °C for 4 min, chilled on ice, 10 U TdT (MBI-Fermentas) added and incubated at 37°C for 30 – 60 min. After inactivation of the tailing enzyme (70°C, 5 min), PCR-Mix I was added consisting of 4 µl of buffer 1 (Roche, Taq long template), 3% deionized formamide (Sigma) in a volume of 35 µl. The probes were heated at 78°C in the PCR cycler (Perkin Elmer 2400), PCR Mix II, containing dNTPs at a final concentration of 350 µM, CP2 primer (5'-TCA-GAA-TTC-ATG-CCC-CCC-CCC-CCC-CCC-3' (SEQ ID NO:[] 27), final concentration 1.2 µM) and 5 Units of the DNA Poly-Mix was added, (Roche, Taq Long Template) in a volume of 5 µl for a hot start procedure. Forty cycles were run at 94°C for 15 sec, at 65°C, 30°C, 68°C for 2 min. for the first 20 cycles and a 10 sec- elongation of the extension time each cycle for the remaining 20 cycles, and a final extension step at 68°C, 7 min

Please replace the paragraph beginning on page 61, line 12 and ending on line 20 with the following paragraph:

Amplification of HA-1 and CD45. All samples were analyzed by two primer pairs for HA-1: HA-1 (I) (forward: 5'-GAC GTC GTC GAG GAC ATC TCC CAT-3' (SEQ ID NO: 17);

reverse: 5'-GAA GGC CAC AGC AAT CGT CTC CAG-3' (SEQ ID NO:[] 18)) and HA- 1 (II) (forward: 5'-ACA CTG CTG TCG TGT GAA GTC-3' (SEQ ID NO:[] 29) reverse: 5'-TCA GGC CCT GCT GTA CTG CA-3' (SEQ ID NO:[] 30)). CD45 forward: 5'- CTG AAG GAG ACC ATT GGT GA (SEQ ID NO:[] 31)) and reverse 5'-GGT ACT GGT ACA CAG TTC GA-3' (SEQ ID NO:[] 32) primer. Amplification products of the HA-1 (I) primers were digested with the restriction enzyme BstU I and amplification products of the HA-1 (II) primers with Hinf I. Southern blot was performed according to standard protocols.

IN THE DRAWINGS:

As requested in the Notice to File Missing Parts, please replace the drawings figures with the replacement figures presented herein. The replacement figures herein should be electronically reproducible.